SUPPLEMENTARY FIGURES

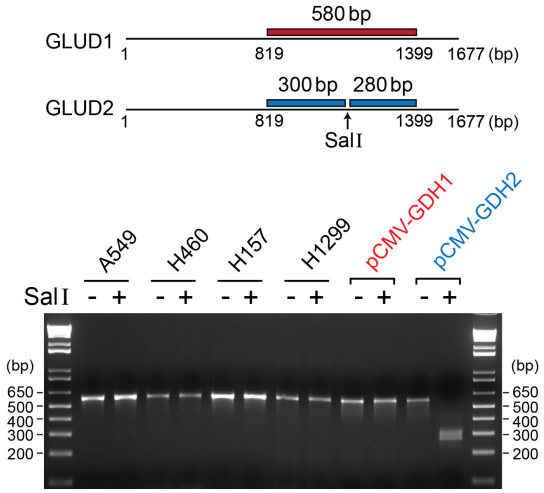

Figure S1, related to Figure 1. GDH1 (GLUD1) is the major isoform expressed in lung cancer cell lines. Schematic diagram shows GDH2 (GLUD2) contains a unique SalI restriction enzyme digestion site that can be utilized to detect GDH2 gene expression by reverse transcription-PCR and SalI digestion. Total RNA was isolated from diverse lung cancer cell lines, as indicated, and subjected to reverse transcription and PCR using primers to amplify a common 580-bp cDNA segment containing a SalI site in GDH2 but not GDH1. The amplified DNA was digested with SalI and visualized on a 2% agarose gel with ethidium bromide staining. The pCMV-GDH1 and pCMV-GDH2 constructs were similarly subjected to PCR and SalI digestion, and used as positive controls. Data is representative of three independent biological experiments.

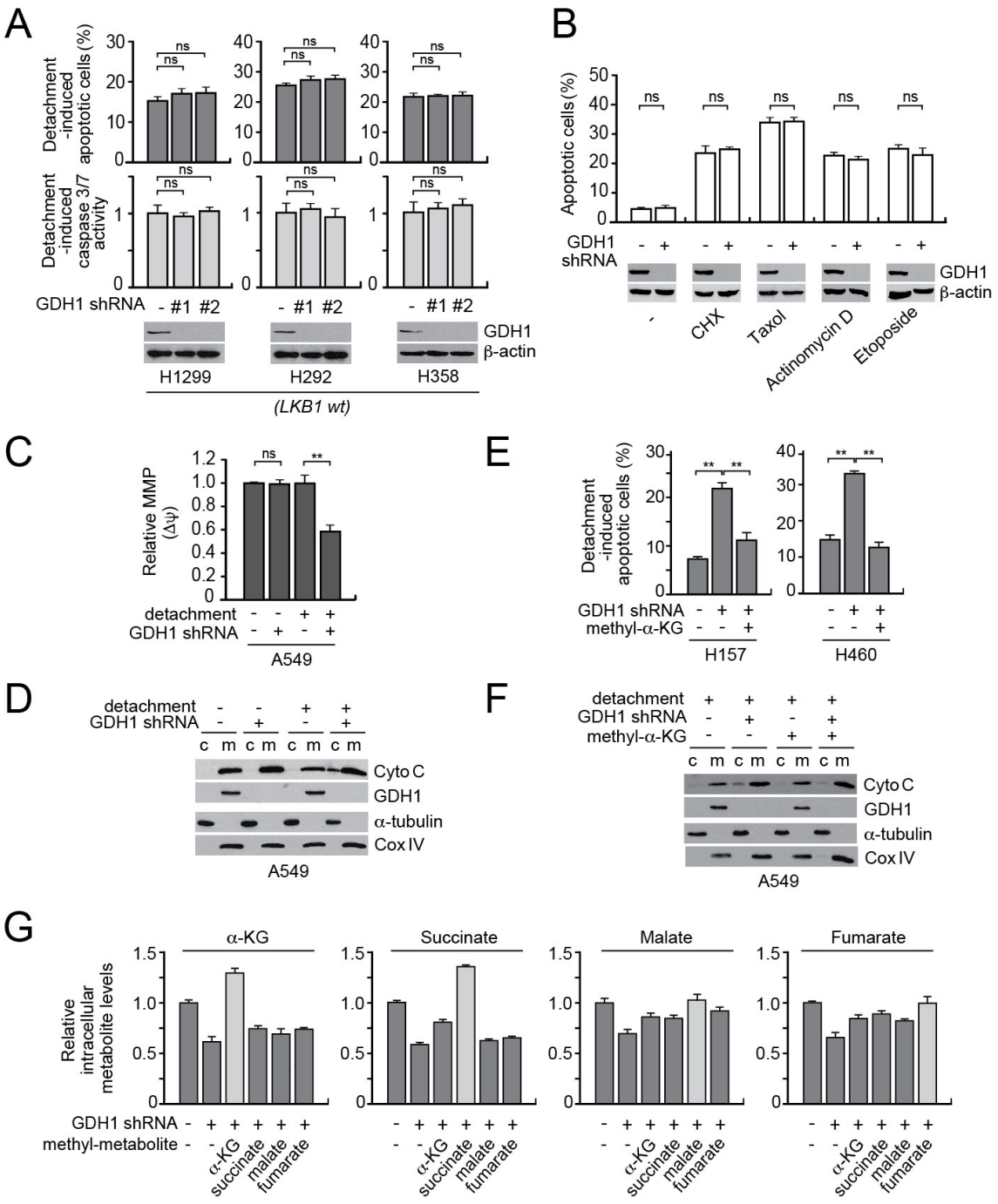

Figure S2, related to Figure 2. Effect of GDH1 loss on apoptosis in lung cancer cells. (A-B) Effect of GDH1 knockdown on apoptosis induced by detachment in LKB1 *wt* cells (A) or cytotoxic agents in LKB1 *null* A549 cells (B). Cells were treated with 5 μg/ml of cycloheximide (CHX), 200 nM taxol (paclitaxel), 5 μM actinomycin D, or 10 μM etoposide for 24 hours. Apoptotic cell death was measured by annexin V staining. **(C-D)** Mitochondrial membrane potential (MMP) and cytochrome C release in GDH1 knockdown cells with detachment. **(E-F)** Rescue effect of α-KG on anoikis resistance and cytochrome C release in GDH1 knockdown cells. **(G)** Intracellular metabolite levels upon methyl-metabolite treatment in detached A549 cells. Data are mean ± SD of three technical replicates and are representative of three (A and E), four (D) or two (B, C, F, G) independent biological experiments. p values were determined by a two-tailed Student's *t* test (ns: not significant; **: $p < 0.01$).

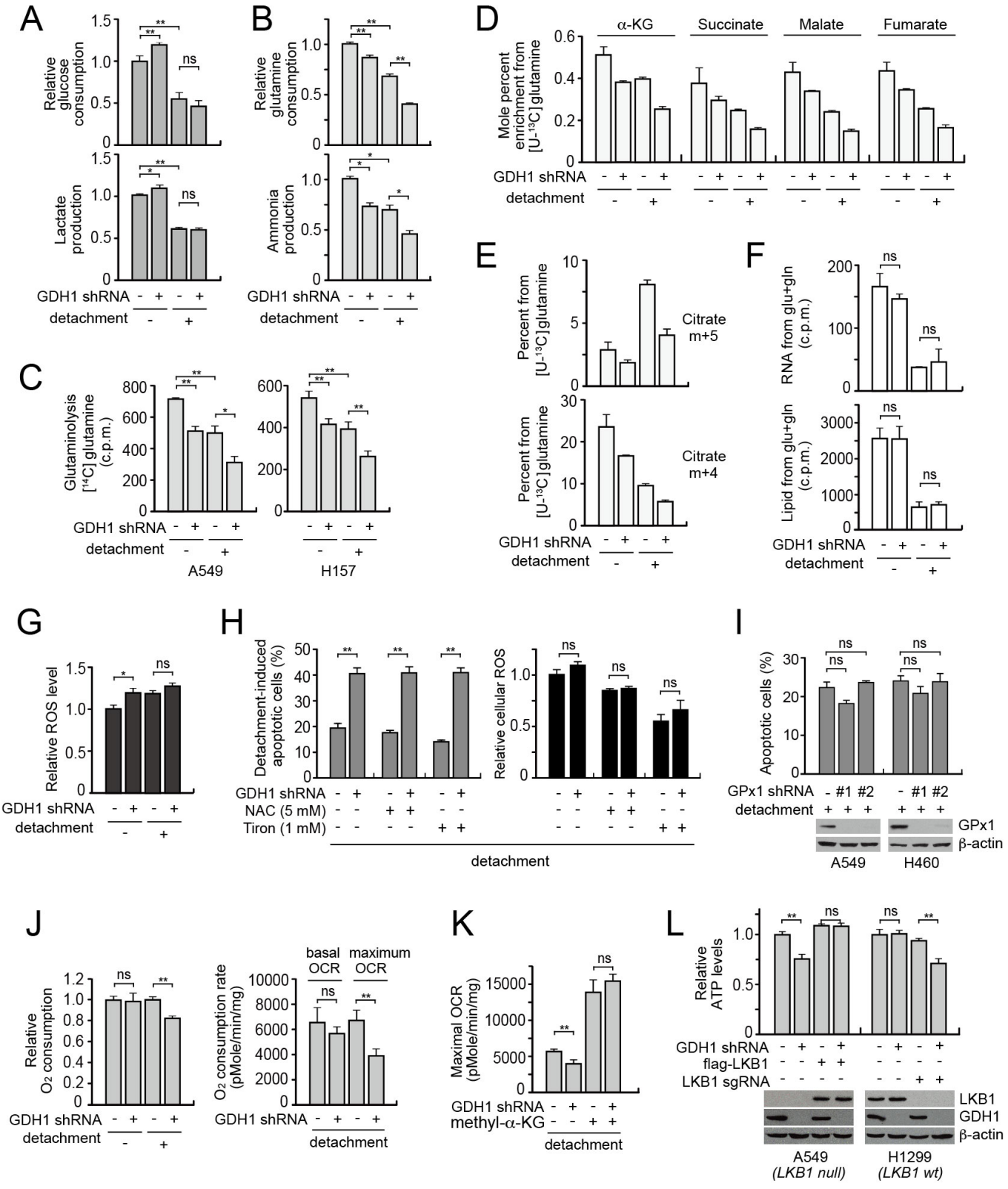

Figure S3, related to Figure 3. GDH1 is essential for supplying α–KG to the TCA cycle and for maintaining energy homeostasis under detached culture conditions in LKB1 null cells. (A-B) A549 cells expressing empty vector or GDH1 shRNA were cultured under attached or detached conditions for 24 hours. The culture medium was collected and levels of glucose (A, *upper*), lactate (A, *lower*), glutamine

(B, *upper*) or ammonia (B, *lower*) were determined using commercial assay kits. (**C**) Glutaminolysis rate was determined using [^{14}C] glutamine in A549 or H157 cells with or without GDH1 knockdown cultured under attached or detached condition for 24 hours. (**D**) Mole percent enrichment (MPE) of metabolites, as indicated, from [U-^{13}C] glutamine in A549 cells with vector or GDH1 shRNA cultured in attached or detached conditions for 24 hours. (**E**) Glutamine reductive carboxylation ([U-^{13}C] glutamine-derived m+5 citrate, *upper*) and oxidation ([U-^{13}C] glutamine-derived m+4 citrate, *lower*) were determined by stable isotope tracing. (**F**) Effect of GDH1 knockdown in A549 cells on RNA and lipid synthesis under attached or detached culture conditions. (**G**) Cellular ROS levels in A549 cells with or without GDH1 knockdown under attached or detached culture conditions. **(H)** Detached A549 cells with or without GDH1 knockdown were treated with antioxidants NAC (5 mM) or tiron (1 mM). Anoikis and cellular ROS levels were determined. **(I)** Effect of GPx1 knockdown on anoikis resistance. A549 (*left*) and H460 (*right*) cells with GPx1 knockdown were cultured under detached condition followed by annexin V staining. (**J**) Effect of GDH1 knockdown on oxygen consumption in A549 cells under attached or detached culture condition. Oxygen consumption rates (OCR) (*left*) and basal/maximum OCR (*right*) were measured by clark-type oxygen electrode and XF24 extracellular flux analyzer, respectively. **(K)** Effect of α-KG on OCR in GDH1 knockdown cells. A549 cells were cultured under detachment conditions with methyl-α-KG, and maximum OCR was determined by XF24 analyzer. (**L**) Effect of GDH1 knockdown on ATP levels in detached A549 cells with or without LKB1 expression or H1299 cells with or without LKB1 knockout. Data are mean ± SD of three technical replicates and are representative of three (G, J) or two (A-C, F, H, I, K, L) independent biological experiments. (D and E) are mean ± SD of two technical replicates from one experiment. p values were determined by a two-tailed Student's *t* test (ns: not significant; *: $0.01 < p < 0.05$; **: $p < 0.01$).

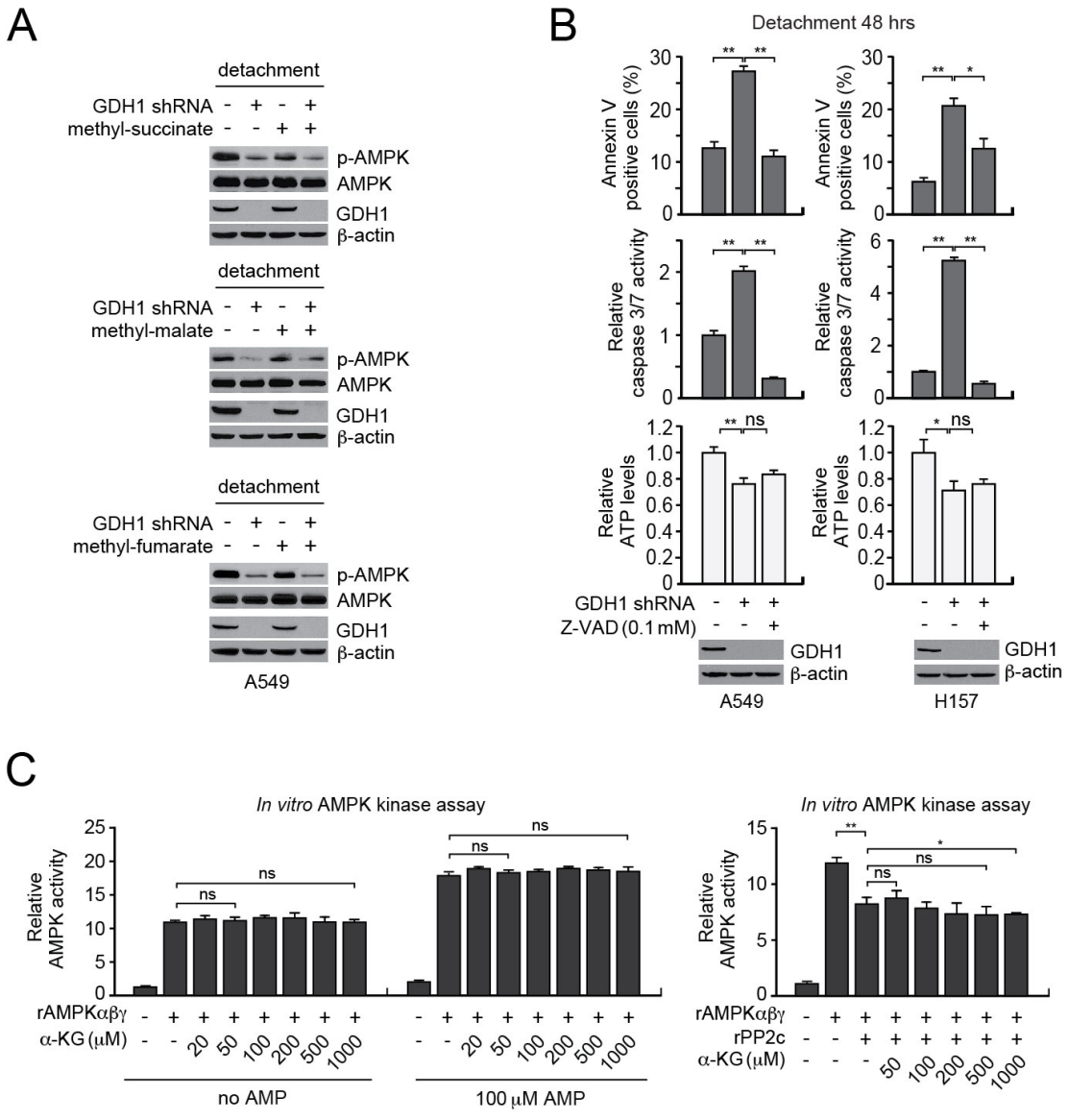

Figure S4, related to Figure 3. GDH1 confers anoikis resistance through AMPK activation and consequent energy regulation. (**A**) Detached A549 cells were cultured in the presence or absence of cell permeable succinate (*upper*), malate (*middle*), or fumarate (*lower*), and AMPK activity was assayed by p-T172 AMPKα western blot. (**B**) Effect of apoptosis inhibition using Z-VAD-FMK on apoptosis (*upper*), caspase 3/7 activity (*middle*) or ATP levels (*lower*) in A549 or H157 cells with or without GDH1 knockdown cultured under detached condition for 48 hours. (**C**) Effect of α-KG on AMPK kinase activity. Activity of recombinant AMPKαβγ in the presence or absence of AMP and different concentrations of α-KG. To test the effect of α-KG on AMPK dephosphorylation, AMPK was incubated with recombinant PP2c in the presence of different concentrations of α-KG. AMPK activity was determined using SAMStide. Data are mean ± SD of three technical replicates and are representative of three (C), two (A) or one (B) independent biological experiments. p values were determined by a two-tailed Student's *t* test (ns: not significant; *: $0.01 < p < 0.05$; **: $p < 0.01$).

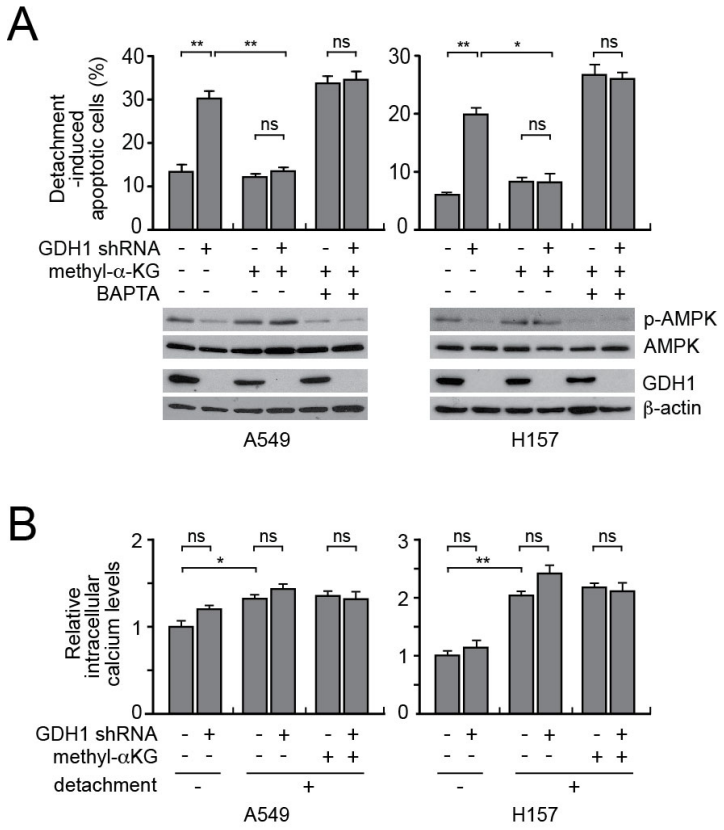

Figure S5, related to Figure 4. GDH1 knockdown or cell permeable α-KG treatment has no impact on cellular calcium level but calcium is required for α-KG to rescue GDH1 knockdown cells under detachment culture condition. (**A**) Detached A549 (*left*) or H157 (*right*) cells with or without GDH1 knockdown were treated with methyl-α-KG and a calcium chelator BAPTA (20 μM) for 48 hours. Anoikis induction and AMPK activation were assessed by annexin V staining and western blot analysis of p-T172 AMPKα, respectively. (**B**) Intracellular calcium levels in A549 (*left*) or H157 (*right*) cells with or without GDH1 knockdown and methyl-α-KG treatment were determined by ratiometric analysis of Fura Red and Fluo-3 staining using flow cytometry. Data are mean ± SD of three technical replicates and are representative of two independent biological experiments. p values were determined by a two-tailed Student's *t* test (ns: not significant; *: $0.01 < p < 0.05$; **: $p < 0.01$).

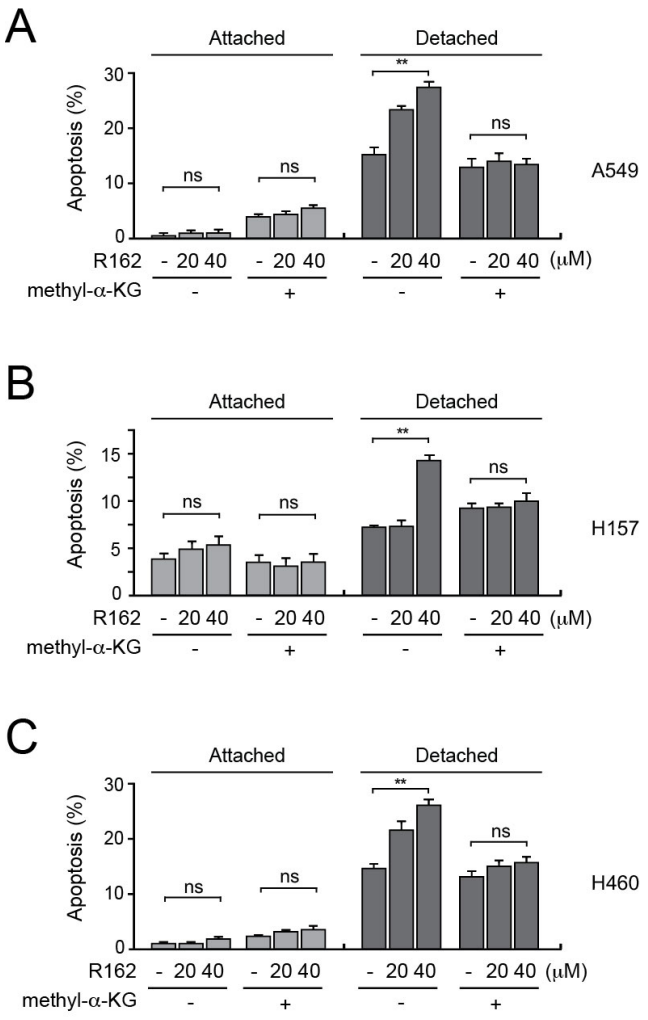

Figure S6, related to Figure 5. Effect of GDH1 inhibition by R162 on apoptosis cultured under attached or detached conditions in diverse LKB1-deficient lung cancer cells. A549 (A), H157 (B) or H460 (C) were cultured under attached or detached conditions in the presence or absence of R162 (20 μM) and methyl-α-KG (5 mM for A549 and H460, 1 mM for H157). Apoptosis was determined by annexin V staining. Data are mean ± SD of three technical replicates and are representative of two independent biological experiments. p values were determined by a two-tailed Student's *t* test (ns: not significant; **: $p < 0.01$).